AF

DOCKET NO.: ORT-1508 (JJPR-0021) **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:
Fredrik Kamme and Jessica Y. Zhu

Application No.: 10/080,795

Filing Date: February 22, 2002

For: Method For Generating Amplified RNA

Confirmation No.: 9944

Group Art Unit: 1637

Examiner: Young J. Kim

DATE OF DEPOSIT: August 30, 2004

I HEREBY CERTIFY THAT THIS PAPER IS BEING DEPOSITED WITH THE UNITED STATES POSTAL SERVICE AS FIRST CLASS MAIL, POSTAGE PREPAID, ON THE DATE INDICATED ABOVE AND IS ADDRESSED TO THE COMMISSIONER FOR PATENTS, P.O. BOX 1450, ALEXANDRIA, VA 22313-1450.

Janet E. Reed

TYPED NAME: Janet E. Reed, Ph.D.
REGISTRATION NO.: 36,252

MS Appeal Brief - Patent
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

APPEAL BRIEF TRANSMITTAL PURSUANT TO 37 CFR § 1.192

Transmitted herewith in triplicate is the APPEAL BRIEF in this application with respect to the Notice of Appeal received by The United States Patent and Trademark Office on **April 5, 2004**.

☐ Applicant(s) has previously claimed small entity status under 37 CFR § 1.27 .

☐ Applicant(s) by its/their undersigned attorney, claims small entity status under 37 CFR § 1.27 as:

- ☐ an Independent Inventor
- ☐ a Small Business Concern
- ☐ a Nonprofit Organization.

09/01/2004 HVUONG1 00000079 10080795
02 FC:1253 950.00 OP

DOCKET NO.: ORT-1508 (JJPR-0021) **PATENT**

☒ Petition is hereby made under 37 CFR § 1.136(a) (fees: 37 CFR § 1.17(a)(1)-(4) to extend the time for response to the Notice of Appeal filed on April 5, 2004 to and through September 5, 2004 comprising an extension of the shortened statutory period of three month(s).

	SMALL ENTITY		NOT SMALL ENTITY	
	RATE	FEE	RATE	FEE
☒ APPEAL BRIEF FEE	$165	$	$330	$330.00
☐ ONE MONTH EXTENSION OF TIME	$55	$	$110	$0.00
☐ TWO MONTH EXTENSION OF TIME	$210	$	$420	$0.00
☒ THREE MONTH EXTENSION OF TIME	$475	$	$950	$950.00
☐ FOUR MONTH EXTENSION OF TIME	$740	$	$1480	$0.00
☐ FIVE MONTH EXTENSION OF TIME	$1005	$	$2010	$0.00
☐ LESS ANY EXTENSION FEE ALREADY PAID	minus	($)	minus	($0.00)
TOTAL FEE DUE		$0		$1,280.00

☒ The Commissioner is hereby requested to grant an extension of time for the appropriate length of time, should one be necessary, in connection with this filing or any future filing submitted to the U.S. Patent and Trademark Office in the above-identified application during the pendency of this application. The Commissioner is further authorized to charge any fees related to any such extension of time to Deposit Account 23-3050. This sheet is provided in duplicate.

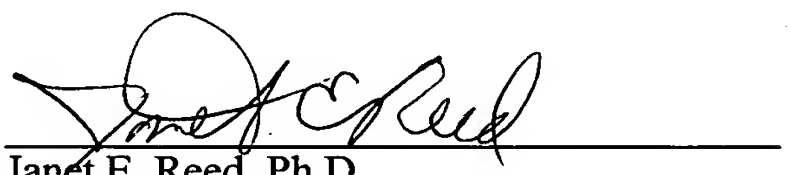
☒ A check in the amount of **$1,280.00**is attached. Please charge any deficiency or credit any overpayment to Deposit Account No. 23-3050.

☐ Please charge Deposit Account No. 23-3050 in the amount of **$ .00**. This sheet is attached in duplicate.

DOCKET NO.: ORT-1508 (JJPR-0021) **PATENT**

☒ The Commissioner is hereby requested to grant an extension of time for the appropriate length of time, should one be necessary, in connection with this filing or any future filing submitted to the U.S. Patent and Trademark Office in the above-identified application during the pendency of this application. The Commissioner is further authorized to charge any fees related to any such extension of time to deposit account 23-3050. This sheet is provided in duplicate.

Date: August 30, 2004



Janet E. Reed, Ph.D.
Registration No. 36,252

Woodcock Washburn LLP
One Liberty Place - 46th Floor
Philadelphia PA 19103
Telephone: (215) 568-3100
Facsimile: (215) 568-3439

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Fredrik Kamme and Jessica Y. Zhu
Confirmation No.: 9944

Serial No.: 10/080,795
Group Art Unit: 1637

Filing Date: February 22, 2002
Examiner: Young J. Kim

For: Method For Generating Amplified RNA

DATE OF DEPOSIT: August 30, 2004

I HEREBY CERTIFY THAT THIS PAPER IS BEING DEPOSITED WITH THE UNITED STATES POSTAL SERVICE AS FIRST CLASS MAIL, POSTAGE PREPAID, ON THE DATE INDICATED ABOVE AND IS ADDRESSED TO THE COMMISSIONER FOR PATENTS, P.O. BOX 1450, ALEXANDRIA, VA 22313-1450.

TYPED NAME: Janet E. Reed
REGISTRATION NO.: 36,252

Mail Stop Appeal-Brief Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

APPELLANT'S BRIEF PURSUANT TO 37 C.F.R. § 1.192

This brief is being filed in support of Appellant's appeal from the final rejections of claims 1-26 in the Office Action dated October 6, 2003. A Notice of Appeal was filed on April 5, 2004 and received at the U.S. Patent and Trademark Office (USPTO) on April 9, 2004. A petition for extension of time for two (2) months through August 5, 2004 and the appropriate fee accompany this brief.

1. REAL PARTY IN INTEREST

The real party in interest in the above-identified patent application is Ortho-McNeil Pharmaceutical, Inc., a corporation of New Jersey, which is the assignee of Fredrik Kamme and Jessica Y. Zhu , as reflected in an assignment recorded in the USPTO on August 26, 2003, at Reel 013912, Frame 0217.

2. RELATED APPEALS AND INTERFERENCES

There are no other appeals or interferences known to Appellant, Appellant's legal representative, or the assignee that will directly affect or be directly affected by or have a bearing on the Board's decision in the present Appeal.

3. STATUS OF CLAIMS

Claims 1-23 are pending in this patent application and are the subject of this appeal. A listing of the claims is provided in Appendix A.

4. STATUS OF AMENDMENTS

By an amendment under 37 C.F.R. § 1.116 dated January 6, 2004, kit claims 24-26 were canceled. The Advisory Action dated January 16, 2004, indicated that the amendment was entered. No subsequent amendments have been filed.

5. SUMMARY OF INVENTION

The claimed invention relates to certain processes for amplifying RNA from cells. In the embodiments of the invention defined by independent claims 1 and 14, a thermostable DNA polymerase is used in the second strand synthesis step (Specification at page 5, lines 3-4).

More particularly, in one general aspect, the invention is directed to a method of amplifying at least one mRNA in a sample containing a plurality of different mRNAs, comprising the following steps:

(a) synthesizing first strand DNA by contacting under conditions conducive to reverse transcriptase activity at least one mRNA in the sample with (i) reverse transcriptase, and (ii) a first primer that is sufficiently complementary to a sequence in the mRNA so as to prime synthesis in a direction toward the 5' end of the mRNA;

(b) synthesizing a second strand of DNA by contacting under conditions conducive to a thermostable DNA polymerase activity, the conditions comprising an incubation temperature of from 45°C to 80°C, the first strand DNA with (i) a thermostable DNA polymerase selected from the group consisting of Bst DNA polymerase large fragment, Bst DNA polymerase native enzyme, Taq DNA polymerase, Pfu DNA polymerase, Tgo DNA polymerase, Phi29 DNA polymerase plus trehalose, T7 DNA polymerase plus trehalose,

Klenow fragment of *E. coli* polymerase plus trehalose, and native *E. coli* DNA polymerase I plus trehalose, and (ii) a thermostable RNase H; and

(c) transcribing resultant amplified DNA into cRNA by contacting the double stranded DNA with an RNA polymerase under conditions conducive to RNA polymerase activity, such that cRNA is produced. See, e.g., Specification at page 14, line 2; page 14, lines 11-19; and page 5, lines 11-28.

In another general aspect, the invention relates to a method for comparing the presence or amount of at least one mRNA of interest in a first sample and in a second sample, the first sample and the second sample each containing a plurality of different mRNAs from one or more cells. The method comprises the steps of:

(a) synthesizing first strand DNA by contacting under conditions conducive to reverse transcriptase activity at least one mRNA in the sample with (i) reverse transcriptase, and (ii) a first primer that is sufficiently complementary to a sequence in the mRNA so as to prime synthesis in a direction toward the 5' end of the mRNA;

(b) synthesizing a second strand of DNA by contacting under conditions conducive to a thermostable DNA polymerase activity, the conditions comprising an incubation temperature of from 45°C to 80°C, the first strand DNA with (i) a thermostable DNA polymerase selected from the group consisting of Bst DNA polymerase large fragment, Bst DNA polymerase native enzyme, Taq DNA polymerase, Pfu DNA polymerase, Tgo DNA polymerase, Phi29 DNA polymerase plus trehalose, T7 DNA polymerase plus trehalose, Klenow fragment of *E. coli* polymerase plus trehalose, and native *E. coli* DNA polymerase I plus trehalose, and (ii) a thermostable RNase H;

(c) transcribing resultant amplified DNA into cRNA by contacting the double stranded DNA with an RNA polymerase under conditions conducive to RNA polymerase activity, such that cRNA is produced;

(d) labeling the cRNA produced in step (c) with a first label;

(e) repeating steps (a)-(d) with the second sample;

(f) labeling the cRNA produced in step (e) with a second label distinguishable from the first label;

(g) detecting or measuring the mRNA of interest in the first sample by contacting the cRNA labeled with the first label with a polynucleotide capable of hybridizing to the cRNA

of the mRNA of interest under conditions conducive to hybridization; and detecting any hybridization that occurs between the polynucleotide and the cRNA;

(h) detecting or measuring the mRNA of interest in the second sample by contacting the cRNA labeled with the second label with the polynucleotide capable of hybridizing to the cRNA of the mRNA of interest under conditions conducive to hybridization; and detecting any hybridization that occurs between the polynucleotide and the cRNA; and

(i) comparing the mRNA of interest detected or measured in the first sample with the mRNA of interest detected or measured in the second sample. See, e.g., Specification at page 14, line 2; page 14, lines 11-19; and page 6, lines 12 to page 7, line 9.

In one preferred embodiment with respect to step (b) of either of the aforementioned methods, the incubation temperature for second strand synthesis may range from 55°C to 70°C (Specification at page 14, line 3). In another preferred embodiment, the conditions for second strand synthesis comprise an incubation time of from one to sixty minutes, or in a further preferred embodiment, the conditions comprise an incubation time of from five to thirty minutes (Specification at page 14, lines 3-5).

In another preferred embodiment pertaining to second strand synthesis, the thermostable DNA polymerase is Bst DNA polymerase large fragment. In another preferred embodiment, the Bst DNA polymerase large fragment is present in a concentration of from 0.012 to 1.3 units/µl and the thermostable RNase H is present in a concentration of from 0.0031 to 1.3 units/µl (Specification at page 14, lines 5-9).

6. ISSUES ON APPEAL

A. Whether the subject matter of claims 1, 2, 4, 5, 7-14 and 16-23 is patentable under 35 U.S.C. §102(e)[1] in view of the disclosure set forth in U.S. patent 6,271,002 B1 to Linsley et a. (referred to herein as "Linsley").

B. Whether the subject matter of claims 3, 6 and 15 is patentable under 35 U.S.C. §103(a) in view of the teachings set forth in U.S. patent 6,271,002 B1 ("Linsley") combined with those of U.S. patent 6,436,677 B1 to Gu et al. (referred to herein as "Gu").

---

1 The final Office Action dated October 6, 2003 states that the rejection based on the Linsley patent was made pursuant to 35 U.S.C. §102(a), rather than §102(e). However, this appears to be an erroneous identification of the applicable provision of Section 102, inasmuch as the Linsley patent's issue date is subsequent to the filing date of the present application.

7. GROUPING OF CLAIMS

In accordance with 37 C.F.R. §1.192(c)(7), Appellants confirm that claims 1-23 do not stand or fall together, for the reasons set forth in the following section.

8. ARGUMENT

The invention as claimed in claims 1, 2, 4, 5, 7-14 and 16-23 is patentable over Linsley because the reference does not identically disclose or suggest all elements or features of the method recited in those claims. The invention as recited in each of claims 3, 6 and 15 is also patentable under 35 U.S.C. §103(a) over Linsley in view of Gu because the references, singly or combined, do not teach or suggest the claimed invention.

A. The Claims Are Patentable Over Linsley. The rejection of claims 1, 2, 4, 5, 7-14 and 16-23 under 35 U.S.C. §102 for alleged anticipation based on Linsley is improper and should be reversed. In rejecting a claim for anticipation, the USPTO carries the burden to establish that "each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California,* 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). The Examiner has failed to satisfy this requirement with respect to the instant rejection under 35 U.S.C. § 102.

Linsley fails to disclose a method as defined in either of independent claims 1 and 14 with respect to at least three elements pertaining to step (b) of the method, second strand DNA synthesis. Namely, Linsley does not disclose a method in which: (1) a second strand of DNA is synthesized by contacting the first strand of DNA with a thermostable DNA polymerase, selected from Bst DNA polymerase large fragment, Bst DNA polymerase native enzyme, Taq DNA polymerase, Pfu DNA polymerase, Tgo DNA polymerase, Phi29 DNA polymerase plus trehalose, T7 DNA polymerase plus trehalose, Klenow fragment of *E. coli* polymerase plus trehalose, and native *E. coli* DNA polymerase I plus trehalose; (2) the second strand of DNA is synthesized at a temperature of from 45°C and 80°C; and (3) the second strand of DNA is synthesized in the presence of a thermostable RNase H.

When referring to second strand DNA synthesis, Linsley discloses the use of non-thermostable DNA polymerases only[2], and incubation with the first DNA strand at 37°C for 45-60 minutes, with the reaction being *terminated* by elevating the temperature to 65°C (Linsley, column 15, line 66, through column 16, line14; and Fig. 1).[3] Nevertheless, citing column 15, line 50-59, and Figure 1 of Linsley, the Examiner asserted that "[s]ince the amplification of the double stranded DNA is subject to amplification via Taq polymerase, *arguably*, the first strand DNA is contacted with Taq polymerase to generate a second strand DNA (or its complement)" and that "Linsley et al. do disclose that the second strand synthesis occurs between 37 and 55 °C" (final Office Action, page 4, emphasis added). Neither of these assertions is accurate.

First, the Linsley disclosures cited by the Examiner do not pertain to the synthesis of a second strand of DNA, which is described in the reference at column 15, line 66, through column 16, line 49.[4] Rather, the use of Taq polymerase is disclosed by Linsley in reference to PCR amplification of double stranded DNA according to methods known in the art (see, e.g., col. 18, lines 62-64). The use of a reaction temperature of between 60 and 90 °C, followed by cooling to a temperature between 37 and 55 °C, as described at col. 15, lines 51-56, refers to the synthesis of a *first* strand, not second strand. When describing the synthesis of second strand DNA, Linsley merely describes the use of conventional DNA polymerases such as *E. coli* DNA polymerase, Klenow fragment of *E. coli* DNA polymerase I, and T4 DNA polymerase (see, e.g., col. 16, lines 11-14; col. 41, lines 27-29) and an incubation temperature such as 37°C (col. 16, lines 5-8). The reaction is terminated by inactivating the Klenow fragment by raising the temperature to 65°C but the second strand synthesis reaction itself is not disclosed as being carried out at an elevated temperature.

---

2 At column 16, lines 11-14, Linsley specifies that "the DNA polymerase used for second strand cDNA synthesis is *E. coli* DNA polymerase I, Klenow fragment of *E. coli* DNA polymerase I, or T4 DNA polymerase", none of which is thermostable in the absence of trehalose.

3 The specification recites: "[S]econd strand cDNA synthesis is carried out by a method known in the art. By way of example but not limitation, an aliquot of the first-strand cDNA reversed transcribed above is mixed with dNTPs (200-250 μM each, final concentration) and 1xKlenow reaction buffer (50 mM TRIS-HCl, pH 8.0/10mM $MgCl_2$/50 mM NaCl) in an final volume of about 20 μl. Second strand cDNA synthesis is accomplished by adding Klenow enzyme (3-4 U/reaction; Life Technologies) with an appropriate primer and incubating the mixture at 37°C for about 45-60 minutes. The Klenow reaction is stopped by incubating the reaction mixture at 65°C for 5-10 minutes to inactivate the enzyme."

4 See Notes 2 and 3 above.

Indeed, in the Advisory Action dated January 16, 2004, the Examiner acknowledged that Linsley disclosed second strand synthesis using a Klenow fragment of *E. coli* DNA polymerase I or T4 DNA polymerase "at 37°C for 45-60 minutes, followed by *inactivation* of Klenow polymerase at 65°C." The Examiner nonetheless asserted that the disclosed methodology fell within the scope of the currently amended claims., The teachings of the reference, however, are to the contrary. As acknowledged by the Examiner, the second strand synthesis disclosed by Linsley was performed using Klenow fragment, *not* a thermostable DNA polymerase as recited in the independent claims, and it was performed at 37°C, *not* at 45°C - 80°C as recited in the independent claims. Additionally, Linsley nowhere discloses the use of a thermostable RNAse H in second strand cDNA synthesis, and the Examiner has never asserted that Linsley discloses this element of the claimed method.[5]

For the above reasons alone, the independent claims patentably define over Linsley. The dependent claims recite additional patentably distinguishing features.

For example, dependent claims 2 and 17 each recites an incubation temperature of from 55°C to 70°C. Such an incubation temperature for second strand synthesis is nowhere taught or suggested by Linsley. Furthermore, claim 12 further specifies that the second strand synthesis reaction is performed by incubating the reaction mixture for 5-30 minutes. The subject matter of this claim is further distinguishing over Linsley, inasmuch as Linsley nowhere discloses or suggests a second strand synthesis employing a 5-30 minute incubation of the reaction mixture.

In summary, from a proper reading of Linsley, there can be no reasonable argument that it identically discloses the presently claimed methods. Accordingly, the rejection of claims 1, 2, 4, 5, 7-14 and 16-23 under 35 U.S.C. §102(e) on the basis of Linsley et al. is in error and should be reversed.

B. The Claims Are Patentable Over Linsley Combined with Gu

The rejection of claims 3, 6 and 15 under 35 U.S.C. §103(a) for alleged obviousness based on Linsley in view of Gu is also in error and should be reversed. In establishing a

---

5 In the Advisory Action, the Examimer alleged that Linsley et al. "describe that RNAse H could be added to dissociate RNA:DNA heteroduplex, which would be applicable to any incubation needing to remove RNA:DNA heteroduplex, which would form during the sub-step (b) of the instant claim." However, this statement is inaccurate inasmuch as Linsley discloses the use of RNAse H *only* in the first strand synthesis step (column 15, lines 55-60). But even assuming, *arguendo*, that Linsley's method included RNAse H in the second strand synthesis, Linsley still does not disclose or suggest using a *thermostable* RNAse H in any step.

prima facie case of obviousness under 35 U.S.C. §103, it is incumbent upon the Examiner to provide a reason why one of ordinary skill in the art would have been motivated to modify a prior art reference or to combine reference teachings to arrive at the claimed invention. *Ex parte Clapp*, 227 U.S.P.Q. 972 (Bd. Pat. App. Int. 1985). To this end, the requisite motivation must stem from some teaching, suggestion or inference in the prior art as a whole or from the knowledge generally available to one of ordinary skill in the art and not from Appellant's disclosure. See for example, *Uniroyal Inc. v. Rudkin-Wiley Corp.*, 5 U.S.P.Q.2d 1434 (Fed. Cir. 1988); and *Ex parte Nesbit*, 25 U.S.P.Q.2d 1817, 1819 (Bd. Pat. App. Int. 1992). For the reasons discussed below, a proper *prima facie* case of obviousness has not been set forth.

Claims 3, 6 and 15 are directed to a method for amplifying at least one mRNA in a sample containing a plurality of different mRNAs or to a method for comparing the presence or amount of at least one mRNA of interest in a first sample and in a second sample, the first sample and the second sample each containing a plurality of different mRNAs from one or more cells, as described above. In the method, the first step (reverse transcription) is carried out under standard conditions, but the second strand synthesis is carried out at an elevated temperature (45-80°C) using the thermostable Bst large fragment DNA polymerase and a thermostable RNAse H. An advantage flowing from the claimed invention as a whole is that use of the elevated temperature and thermostable enzymes in the second strand synthesis results in the generation of relatively large amounts of RNA from a small starting number of cells with high efficiency, and in a substantially reduced time period compared to known methods for performing RNA amplification (Specification at page 5, lines 4-8).
Linsley, on the other hand, teaches an RNA amplification method that purports to achieve a high degree of amplification and simultaneously avoid the infidelity associated with respect to sequence and representation that can be introduced by large numbers of PCR cycles (column 3, lines 31-46). In contrast to the claimed method, the method taught by Linsley focuses on combining *in vitro* transcription (IVT) with PCR to achieve the desired result (column 4, lines 5-9) (steps that are performed *after* the double-stranded cDNA has been generated), and nowhere does Linsley teach or suggest modifying the temperature of the reverse transcription or second strand synthesis reactions or using a thermostable Bst enzyme in those reactions. The Examiner therefore looked to Gu for its teaching that the Bst enzyme may be used to catalyze the synthesis of the second strand (column 19, lines 54-57).

However, the Examiner has failed to cite any suggestion in Gu for modifying the second strand synthesis of Linsley so that it is carried out at an elevated temperature and employs a thermostable RNAse H. Thus, even assuming *arguendo* that one of ordinary skill in the art would have selectively picked the Bst enzyme of Gu for use in the second strand synthesis of Linsley, there would have been no motivation to further modify Linsley's method so as to arrive at the invention defined by claims 3 and 15, let alone that defined by claim 6. Accordingly, the teachings of Linsley combined with Gu cannot be said to render any claim obvious.

In sum, even the combined teachings of Linsley and Gu fail to render the claimed invention as a whole obvious. Accordingly, the rejection of claims 3, 6, and 15 under 35 U.S.C. §103(a) on the basis of Linsley and Gu is in error, and should be reversed.

C. Conclusion

Both rejections of the pending claims are improper and should be reversed. For the reasons given above, the claims presented on appeal are directed to patentable subject matter and therefore should be allowed.

Respectfully submitted,

Date: August 30, 2004

Janet E. Reed, Ph.D.
Registration No. 36,252

Woodcock Washburn LLP
One Liberty Place - 46th Floor
Philadelphia PA 19103
Telephone: (215) 568-3100
Facsimile: (215) 568-3439

APPENDIX A TO APPELLANT'S APPEAL BRIEF

The foregoing constitutes a complete listing of the claims on appeal.

1. (previously presented) A method for amplifying at least one mRNA in a sample containing a plurality of different mRNAs comprising:
   a) synthesizing first strand DNA by contacting under conditions conducive to reverse transcriptase activity at least one mRNA in said sample with (i) reverse transcriptase, and (ii) a first primer that is sufficiently complementary to a sequence in the mRNA so as to prime synthesis in a direction toward the 5' end of the mRNA;
   b) synthesizing a second strand of DNA by contacting under conditions conducive to a thermostable DNA polymerase activity, said conditions comprising an incubation temperature of from 45°C to 80°C, the first strand DNA with (i) a thermostable DNA polymerase selected from the group consisting of Bst DNA polymerase large fragment, Bst DNA polymerase native enzyme, Taq DNA polymerase, Pfu DNA polymerase, Tgo DNA polymerase, Phi29 DNA polymerase plus trehalose, T7 DNA polymerase plus trehalose, Klenow fragment of *E. coli* polymerase plus trehalose, and native *E. coli* DNA polymerase I plus trehalose, and (ii) a thermostable RNase H; and
   c) transcribing resultant amplified DNA into cRNA by contacting the double stranded DNA with an RNA polymerase under conditions conducive to RNA polymerase activity, such that cRNA is produced.

2. (previously presented) The method of claim 1 wherein said incubation temperature is from 55°C to 70°C.

3. (previously presented) The method of claim 2 wherein said thermostable DNA polymerase is Bst DNA polymerase large fragment.

4. (previously presented) The method of claim 1 wherein said conditions further comprise an incubation time of from one to sixty minutes.

5. (previously presented) The method of claim 1, which further comprises labeling the transcribed cRNA with a fluorescent, radioactive, enzymatic, hapten, biotin, or digoxigenin label.

6. (previously presented) The method of claim 1, wherein the thermostable DNA polymerase is Bst DNA polymerase large fragment present in a concentration of from 0.012 to 1.3 units/µl and the thermostable RNase H is present in a concentration of from 0.0031 to 1.3 units/µl.

7. (previously presented) The method of claim 5, wherein the label is fluorescent.

8. (original) The method of claim 7 wherein the fluorescent label is fluorescein isothiocyanate, lissamine, Cy3, Cy5, or rhodamine 110.

9. (original) The method of claim 7, wherein a first aliquot of the cRNA is labeled with a first fluorophore having a first emission spectrum, and a second aliquot of the cRNA is labeled with a second fluorophore with a second emission spectrum differing from that of the first emission spectrum.

10. (original) The method of claim 9, wherein the first fluorophore is Cy3 and the second fluorophore is Cy5.

11. (original) The method of claim 1 further comprising, after the transcribing step, determining the presence or absence of a pre-selected target mRNA in said sample.

12. (previously presented) The method of claim 1, wherein the conditions further comprise an incubation time of from five to thirty minutes.

13. (original) The method of claim 1, wherein the mRNA is extracted from at least one cell of interest, and further comprising contacting the cRNA produced in step (d) with an array containing one or more species of polynucleotide positioned at pre-selected sites on the array, under conditions conducive to hybridization; and detecting any hybridization that occurs between said one or more species of polynucleotide and said cRNA.

14. (previously presented) A method for comparing the presence or amount of at least one mRNA of interest in a first sample and in a second sample, said first sample and said second sample each containing a plurality of different mRNAs from one or more cells, comprising:

a) synthesizing first strand DNA by contacting under conditions conducive to reverse transcriptase activity at least one mRNA in said first sample with (i) reverse transcriptase, and (ii) a first primer that is sufficiently complementary to a sequence in the mRNA so as to prime synthesis in a direction toward the 5' end of the mRNA;

b) synthesizing a second strand of DNA by contacting under conditions conducive to thermostable DNA polymerase activity, said conditions comprising an incubation temperature of from 45°C to 80°C, the first strand DNA with (i) a thermostable DNA polymerase selected from the group consisting of Bst DNA polymerase large fragment, Bst DNA polymerase native enzyme, Taq DNA polymerase, Pfu DNA polymerase, Tgo DNA polymerase, Phi29 DNA polymerase plus trehalose, T7 DNA polymerase plus trehalose, Klenow fragment of *E. coli* polymerase plus trehalose, and native *E. coli* DNA polymerase I plus trehalose, and (ii) a thermostable RNase H;

c) transcribing resultant double stranded DNA into cRNA by contacting the amplified DNA with an RNA polymerase under conditions conducive to RNA polymerase activity, such that cRNA is produced;

d) labeling the cRNA produced in step (c) with a first label;

e) repeating steps (a)-(d) with said second sample;

f) labeling the cRNA produced in step (e) with a second label

distinguishable from said first label;

g) detecting or measuring the mRNA of interest in the first sample by contacting the cRNA labeled with said first label with a polynucleotide capable of hybridizing to said cRNA of the mRNA of interest under conditions conducive to hybridization; and detecting any hybridization that occurs between said polynucleotide and said cRNA;

h) detecting or measuring the mRNA of interest in the second sample by contacting the cRNA labeled with said second label with said polynucleotide capable of hybridizing to said cRNA of the mRNA of interest under conditions conducive to hybridization; and detecting any hybridization that occurs between said polynucleotide and said cRNA; and

i) comparing the mRNA of interest detected or measured in said first sample with the mRNA of interest detected or measured in said second sample.

15. (previously presented) The method of claim 14 wherein said thermostable DNA polymerase is Bst DNA polymerase large fragment.

16. (previously presented) The method of claim 14 wherein said sample contains total RNA or total mRNA from mammalian cells.

17. (currently amended) The method of claim 14 wherein said the incubation temperature is from 55°C to 70°C.

18. (original) The method of claim 14 wherein said first label is Cy3 and said second label is Cy5.

19. (original) The method of claim 14 wherein said detecting or measuring steps (g) and (h) are carried out by a method comprising contacting said cRNA with an array containing one or more species of polynucleotide positioned at pre-selected sites on

the array, under conditions conducive to hybridization; and detecting any hybridization that occurs between said polynucleotide and said cRNA.

20. (original) The method of claim 14 wherein the array comprises a support with at least one surface and more than one different polynucleotides, each different polynucleotide comprising a different nucleotide sequence and being attached to the surface of the support in a different, selected location on said surface.

21. (original) The method of claim 14 wherein the array has at least 1,000 polynucleotide probes per square centimeter.

22. (original) The method of claim 14 wherein in steps (g) and (h), the steps of contacting the cRNA labeled with said first label with said polynucleotide probe, and contacting the cRNA labeled with said second label with said polynucleotide probe, are carried out concurrently.

23. (original) The method of claim 14 wherein said first sample contains mRNAs from cells that are pathologically aberrant and wherein said second sample contains mRNAs from cells that are not pathologically aberrant.

24. (canceled).

25. (canceled).

26. (canceled).